Research paper

Chronic 5-HT₄ receptor agonist treatment restores learning and memory deficits in a neuroendocrine mouse model of anxiety/depression

Flavie Darcet, Alain M. Gardier, Denis J. David*, Jean-Philippe Guilloux*

Université Paris-Saclay, Univ. Paris-Sud, Faculté de Pharmacie, CESP, INSERM UMR5178, Chatenay-Malabry 92296, France

HIGHLIGHTS

• A chronic corticosterone administration induces emotional and cognitive impairments.
• Chronic fluoxetine only partially reversed these cognitive alterations.
• A 5-HT₄ receptor agonist restored CORT-induced deficits in all cognitive domains.

ABSTRACT

Cognitive disturbances are often reported as serious invalidating symptoms in patients suffering from major depression disorders (MDD) and are not fully corrected by classical monoaminergic antidepressant drugs. If the role of 5-HT₄ receptor agonists as cognitive enhancers is well established in naïve animals or in animal models of cognitive impairment, their cognitive effects in the context of stress need to be examined. Using a mouse model of anxiety/depression (CORT model), we reported that a chronic 5-HT₄ agonist treatment (RS67333, 1.5 mg/kg/day) restored chronic corticosterone-induced cognitive deficits, including episodic-like, associative and spatial learning and memory impairments. On the contrary, a chronic monoaminergic antidepressant drug treatment with fluoxetine (18 mg/kg/day) only partially restored spatial learning and memory deficits and had no effect in the associative/contextual task. These results suggest differential mechanisms underlying cognitive effects of these drugs. Finally, the present study highlights 5-HT₄ receptor stimulation as a promising therapeutic mechanism to alleviate cognitive symptoms related to MDD.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Increasing evidence suggests that cognitive disturbances are closely associated with depressive symptoms observed in major depression disorders (MDD) [1,2]. In addition to the emotional and behavioral alterations characterizing this pathology, patients suffering from MDD display a broad range of cognitive deficits, varying from executive functions, attention, processing speed, working memory to visual learning and memory domains [3,4]. Cognitive processes such as attention and memory may not only be correlates of depressive episodes; they may also play a critical role in increasing individuals' vulnerability for the first onset and recurrence of depression (for review see Ref. [5]). Currently, these cognitive signs are not fully corrected by classical monoaminergic antidepressant drugs [6,7]. New antidepressant drug strategies that target mood-related symptoms as well as cognitive symptoms are needed to improve long-term outcomes, and particularly functional recovery [8].

It has been proposed that not only 5-HT₄ receptor agonists such as RS67333 may bring new hope for treating depression [9,10] for review), but also represent a promising drug candidate to treat cognitive impairments [11]. During the past few years, the role of 5-HT₄ receptor agonists as cognitive enhancers has been well-established under basal conditions in rodents [12,13], in young or aged macaques [14] or in animal models of cognitive impairment [11,15,16], but their pro-cognitive effects in the context of anxiety/depression-like state has not been examined yet. A variety of studies have assessed cognitive disorders in different anxiety...
or depression models in adult rodents [17–19]. Specifically, we previously showed that chronic corticosterone exposure in mice (CORT model) induces an anxiety/depressive-like phenotype [20], associated with episodic-like, associative and spatial learning and memory impairments [21]. Moreover, sub-chronic and chronic 5-HT4 receptor stimulations reversed the CORT-induced higher emotional state [9,22]. The aim of the present study was to determine whether chronic activation of the 5-HT4 receptor (RS67333), in comparison to fluoxetine, a chronic serotonergic antidepressant drug treatment, could reverse CORT-induced cognitive deficits. To this end, we selected a range of cognitive behavioral paradigms that allows investigation of different memory systems including episodic-like memory, associative/contextual memory and spatial reference learning and memory.

2. Materials and methods

2.1. Animals

Eight to 10-weeks old male C57BL/6J mice (Janvier Labs, France) were maintained on a 12L:12D schedule (Lights On at 7AM) and were housed 5 per cage. Food and water were provided ad libitum. All testing were conducted in compliance with the laboratory animal care guidelines, with protocols approved by the Institutional Animal Care and Use Committee (CEEB authorization 2012–099) and with the European directive 2010/63/EU.

2.2. Drugs and treatments

Corticosterone (CORT, 4-pregnen-11b-DIOL-3 20-DIONE 21-hemisuccinate (35 µg/ml equivalent to 5 mg/kg/day) from Sigma-Aldrich (France) was dissolved in vehicle (0.45% hydroxypropyl-β-cyclodextrin, β-CD), Sigma-Aldrich, France). Fluoxetine hydrochloride (Anawa trading, Switzerland) was dissolved in 0.45% β-CD [20]. RS67333 hydrochloride (1-(4-amino-5-chloro-2-methoxyphenyl)-3-(1-butyl-4piperidinyl)-1-propanone) (Tocris Bioscience, United Kingdom) was dissolved in 0.9% saline solution. Corticosterone was delivered for 28 days in drinking water to induce the anxi-depressed phenotype in mice (Supplementary Fig. 1). Thereafter, while administration with β-CD or corticosterone continued, mice were treated with vehicle (0.45% β-CD), fluoxetine (160 mg/ml delivered in drinking water, equivalent to 18 mg/kg/day) or RS67333 (delivered by osmotic mini-pumps at a dose of 1.5 mg/kg/day). The dose, route and duration of fluoxetine or RS67333 treatments were selected based on our previous report [9], to ensure a similar anxiolytic/antidepressant-like effect of treatment. Osmotic minipumps (42 days, model 2006, Alzet, USA) were implanted subcutaneously under light anesthesia (ketamine/xylazine: 75/20 mg/kg, Sigma Aldrich, France). Vehicle, CORT and CORT/Fluoxetine groups were also implanted with a mini-pump containing 0.9% saline. Treatment was maintained until the end of the experiments.

2.3. Behavioral testing

Cognitive and anxi/depressive effects of pharmacological treatments were conducted within the same cohort. Each animal was successively subjected to the behavioral tests as shown in Supplementary Fig. 1. At least one day resting was given to animals between each test. Animals were placed in the experimental room 30 min before the start of the behavioral experiments. A detailed description of materials and methods can be found in supplemental information.

2.3.1. Anxiety and depression behavior paradigms

Anxiety parameters and motor activity were quantified during 10 or 30 min respectively in Open Field (OF) boxes as described in [9]. Depressive-like behavior was assessed in the Splash test (ST) as previously described [20].

2.3.2. Cognition behavioral paradigms

2.3.2.1. Episodic memory: novel object recognition test (NORT). The procedure was adapted from the Sahay study [23] and was performed as previously described in [21]. Briefly, the NORT was divided into 4 training sessions and one test session. During training sessions, two identical objects were present in the box and the mouse was allowed to freely explore the apparatus and the objects. Results for this test were expressed as: 1) exploration (in percent) of each object during the test session and 2) a discrimination index (DI) between objects during the test session, calculated as the difference between the time spent exploring the novel object (N) and the familiar object (F) divided by the total time exploring both objects (DI = (N − F)/(N + F)).

2.3.2.2. Associative/contextual memory: one-trial contextual fear conditioning. Associative/contextual memory was evaluated as previously described in [21], adapted from previous studies [24]. The experimental design ran over two consecutive days. On Day 2, animals returned to the conditioning chamber for a 4 min period, for a test of context-elicted freezing. Scoring was measured using Freezing software version 2.0.4 (Packwin, Harvard apparatus, Bioseb, France).

2.3.2.3. Spatial reference learning and memory: Barnes maze. The Barnes maze (BM) procedure was conducted as previously described [21]. Briefly, spatial acquisition was organized in 4 training sessions (Day 1 to Day 4) followed on day 5 by a retention probe trial (90 s) during which the target box was removed and the target hole was closed. Primary latency and primary errors to identify the target hole were manually scored. Latency to reach the target hole for the first time, number of errors before reaching the target hole, number of visits in the target hole and time spent in the target quadrant were recorded using ANY-maze Software version 4.99 (Stoelting, Bioseb, France).

2.4. Statistics

Results from data analyses were expressed as mean ± SEM. Statistical analyses were processed with Statview 5.0® Software (SAS Institute, Cary, NC). Detailed statistical methodology and results are provided in Supplemetnals.

3. Results

In the present study, we confirmed previous reports showing chronic corticosterone-induced anxiety/depression-like phenotype in mice [20]. Physiological (weight) and physical (fur coat) changes are indicators of depressed-like state in animals: here, a significant increase in body weight and coat state score in CORT-treated animals compared to controls was observed (p < 0.01, Fig. S2A and B). Moreover, CORT-treated mice displayed anxiety- and depressive-like phenotypes in the OF and the ST compared to controls. Interestingly, both chronic fluoxetine and 5-HT4 receptor activation with RS67333 were able to significantly reverse chronic CORT-induced increase in body weight and deterioration of coat state (p < 0.05 and p < 0.01). In the OF, a trend and a significant increase in time spent in the center were observed in chronic R67333 or fluoxetine-treated mice, respectively (p = 0.09; p < 0.05), without affecting the total ambulatory distance (p > 0.1) (Fig. S2C and D). In the ST, both pharmacological treatments significantly
reversed the chronic CORT treatment-induced decrease in grooming duration (p < 0.01) (Fig. S2E). Altogether, these data confirmed that 5-HT4 receptor activation produces both anxiolytic-like and antidepressant-like effects comparable to those of fluoxetine in the chronic CORT model [9].

3.1. Effects of chronic fluoxetine or 5-HT4 receptor agonist treatment on episodic-like memory in mice chronically treated with corticosterone

In the NORT, all experimental groups were able to discriminate the novel object from the familiar one, indicated by a significant increase in relative exploration time (p < 0.01 versus the chance level 50% for each group, Fig. 1A). However, CORT-treated animals displayed a lower distinction for the novel object than vehicle animals, as suggested by a decrease in discrimination index (Fig. S3A, see also Ref. [21]). Both chronic fluoxetine or RS67333 treatments reversed CORT-induced episodic-like impairment by increasing novel object exploration time and consequently increasing the discrimination index (p < 0.01, Figs. 1A and S3A). No significant difference was found between groups on control parameters (exploration duration across sessions and ambulatory distances) (p > 0.1, Figs. 1B and S3A–C).

3.2. Effects of chronic fluoxetine and 5-HT4 receptor agonist treatment on associative/contextual memory in mice chronically treated with corticosterone

During the Day 2 trial, CORT-treated mice exhibited a lower freezing duration than controls, associated with a decrease in freezing episodes (p < 0.01, Fig. 1C and D). RS67333 treatment restored CORT-induced associative/contextual memory deficit, by increasing freezing time and episodes (p < 0.05, Fig. 1C and D). On the contrary, fluoxetine failed to improve the freezing duration during the context-elicited fear trial and even showed a decrease in freezing episodes compared to CORT-treated mice (p < 0.05, Fig. 1D).
3.3. Effects of chronic fluoxetine and 5-HT4 receptor agonist treatment on spatial learning and memory performances in mice chronically treated with corticosterone

Motivational behavior to move and search for the target hole was controlled by measuring mean speed during the training session of Day 1, and did not differ between groups (p > 0.1) (Fig. S4D).

3.3.1. Acquisition

Learning occurred in all groups, as latency to identify the target hole was decreased across learning sessions (p < 0.01, Fig. 2A). However, an increase in primary latency and errors in CORT-treated mice compared to controls confirmed the altered spatial learning performances in these animals compared to controls (p < 0.01, Figs. 2A and S4C, see also [21]). Interestingly, both CORT/Fluox and CORT/RS67333 animals were able to partially recover learning abilities, as indicated by a progressive significant decrease in primary latency across time compared to CORT-treated mice (p < 0.01, Fig. 2A). Paradoxically, while CORT/RS67333 showed a classical simultaneous decrease in primary errors across trials (p < 0.01), CORT/Fluox animals failed to improve this parameter, suggesting that research strategy of the target hole is different between treatments (Fig. S4A and B).

3.3.2. Retention trial

On Day 5, reference memory was assessed by a retention trial in which the target hole was removed and replaced by a false hole. Increases in primary errors and primary latency in CORT-treated mice compared to controls (p < 0.01, Fig. 2B and C) associated with a decrease in time spent in the target quadrant (p < 0.05, Fig. 2D) and a decrease in the number of visits in the target hole (p < 0.01, Fig. 2E) suggested that anxious/depressive-like animals have difficulties to remember the location of the target quadrant. Interestingly, a chronic RS67333 treatment improved all of the retention parameters when compared to CORT-treated mice (p < 0.01, Fig. 2B–E), suggesting that a chronic 5-HT4 receptor stimulation can restore CORT-induced learning and retention spatial deficits. Intriguingly, whereas learning performances were reversed after a chronic fluoxetine treatment, most of the retention parameters remained impaired in CORT/Fluox animals (Fig. 2B–D), except the number of visits into the target hole (p < 0.05, Fig. 2E).

4. Discussion

The present study reveals the cognitive consequences of chronic fluoxetine or 5-HT4 receptor agonist treatments in a neuroendocrine-based mouse model of anxiety/depression. Because memory functions are not limited to a single aspect, episodic-like (novel object recognition test), associative/contextual (one-trial contextual fear conditioning) and spatial (Barnes maze) learning and memory tasks were tested. Our results highlight that altered emotional phenotype after chronic CORT treatment induced a cognitive deficit that affects all aspects of learning and memory, especially episodic (NORT), associative/contextual (CFC) and visuo-spatial systems in mice (BM) [21]. A chronic 5-HT4 receptor agonist treatment restored all the cognitive CORT-induced deficits. By contrast, a chronic fluoxetine treatment was able to reverse deficits in episodic-like memory (NORT) and some of learning parameters in the Barnes maze, but failed to improve the CORT-induced decrease in freezing behavior in the associative/contextual test and most of retention parameters in a spatial task.

The majority of preclinical research focused on the effects of 5-HT4 receptor agonists in the cognitive domain (acute: [12,25]; chronic: [13]). Likewise, numerous studies described RS67333 as a potential antidepressant drug [9,22,26,27], and that fluoxetine antidepressant effects are blocked by a 5-HT4 receptor antagonist, GR125487 [9]. This is the first report of the cognitive effects of a chronic systemic RS67333 treatment in a model of anxiety/depression. Identification of neuronal circuits involved in the cognitive effects elicited by activation of 5-HT4 receptor under anxiety/depression-like phenotype still need further investigations. Although RS67333 displays a partial agonist activity on 5-HT4 receptors, previous studies showed its similar behavioral effects compared to other full receptor agonists [16,26]. Additionally, the agonist efficacy of a drug also depends on the system in which it is evaluated as the receptor density or coupling efficiency can differ [28]. RS67333 shows high binding affinity for the 5-HT4 receptor with a pKi of 8.7 [29,30]. Except for the sigma receptors, which are bound at affinities comparable to 5-HT4 receptors (σ1: pKi = 8.9; and σ2: pKi = 8.0), RS67333 has a pKi of less than 6.7 for other neurotransmitters’ receptors [31]. While we cannot rule out a role of the sigma receptor, GR125487 a 5-HT4 receptor antagonist with no sigma receptor affinity blocked the RS67333-induced preclinical effects [32,33], supporting our hypothesis that 5-HT4 receptor is prominently implicated in the modulation of mood and cognitive behaviors of RS67333.

In the novel object recognition test, RS6733 and fluoxetine had similar effects. Interestingly, neurochemical and behavioral studies suggest that 5-HT4 receptors may play an important role in cognition processes through an interaction between the cholinergic and/or histaminergic systems in the hippocampus or in the cortical areas ([11,34,35] for review). Stimulation of 5-HT4 receptor increases extracellular histamine and acetylcholine levels in rodents in these brain regions involved in cognitive function. In bullectomized mice, a mouse model of depression, chronic fluoxetine was able to restore normal exploratory behavior in the novel object test. In this model in which a 2.5 fold increase in corticosterone levels in serum was observed, chronic fluoxetine was also able to reverse the stress-induced increase in hippocampal acetylcholine esterase (AChE), but also to reduce AChE activity in the prefrontal cortex, corroborating the notion that this SSRI modulates cholinergic neurotransmission [36]. These cholinergic mechanisms may participate to chronic RS67333 and/or fluoxetine-reversed corticosterone induced behavioral deficit in the novelty in the novel object.

In the present study, differential fluoxetine and RS67333 behavioral cognitive effects also rely on different mechanisms of action characterizing these drugs. Whereas RS67333 selectively stimulates 5-HT4 receptors [29], fluoxetine acts as an indirect 5-HT receptors’ agonist, by selectively inhibiting serotonin re-uptake. Beneficial as well as detrimental cognitive effects of fluoxetine may thus involve other serotoninergic receptors, which play a crucial role in learning and memory processes [14,37,38]. For instance, current strategies blocking 5-HT1A, 5-HT3 and 5-HT7 receptors have been shown to improve cognition [13,39,40]. In regards to the detrimental effects of fluoxetine, a recent report using the novel object recognition, suggest that 5-HT2A receptors activation is involved. Indeed, fluoxetine (10 mg/kg i.p.) impaired mice performance test 24 h post-administration [41]. Multiple medication therapy, based on the modulation of these receptors, may represent a more efficient strategy than the use of each drug alone to alleviate cognition deficits in MDD.

Preclinical evidences for cognitive efficacy of SSRIs in animal models of depression are inconsistent (See [42] for review). Effects of chronic fluoxetine on stress-induced memory impairments vary according to the nature of the task and the chronic stress procedures. For example, stress-induced deficits in episodic-like memory was reversed [43] or unchanged in the recognition task [44] following a chronic fluoxetine treatment. Depending on the spatial navigation task, a chronic fluoxetine treatment produced either reversal in deficits [45,46], no effect in the Morris water maze or
impairment in the Radial arm water maze [47]. In our study using the Barnes Maze, opposing results regarding learning parameters (latency and errors) after a chronic fluoxetine treatment reopen the question whether mice actually used a spatial strategy to solve the task [48]. Here, fluoxetine-treated mice adopted a “serial” search strategy, by randomly choosing a hole and visiting adjacent holes until they found the target hole (see Fig. S4A and B). This suggests that these mice correctly learned the rule (since their latency to identify the hole decreased over time), but did not use, or partially, the spatial visual cues to locate the target hole.
To our knowledge, no study has focused on the effects of fluoxetine in the contextual fear-conditioning task in animal models of depression. Indeed, most of the studies investigating effects of SSRIs in conditioned fear response were conducted in naïve animals (see Ref. [49] for review). Following sub-chronic or chronic SSRI treatments in rats, freezing behavior was found to be decreased [50–52] or unchanged [53] compared to controls. Interestingly, Karpova et al. [54] showed that a 3-weeks fluoxetine treatment did not change fear-conditioning behavior, but had a positive synergistic effect when combined with extinction episodes to lose fearful memories. Additionally, chronic citalopram (another SSRI) treatment has been reported to impair the acquisition of contextual fear conditioning in naïve rats because of its anxiolytic-like activity [52]. A direct role of the NR2B subunit in the amygdala in mediating these effects, i.e., a downregulation of the NR2B subunit, has been suggested [49]. Similar mechanism cannot be ruled out for fluoxetine since anxiolytic-like effects have been observed in our study and elsewhere in the CORT model [9,20]. Nevertheless, additional studies are needed to understand the link between SSRIs and fear conditioning in a pathologically stressful context.

From a clinical point of view, several studies support the idea that monoaminergic antidepressant drugs may ameliorate the cognitive functions of depressed patients. Specifically, fluoxetine was found to improve memory and mental processing speed [55,56]. However, some studies did not observe any improvement in cognitive functions after antidepressant drug treatment in depressed patients [6,57], or that medication is ineffective without additional psychotherapeutic intervention [7].

By restoring all of the CORT-induced cognitive deficits as well as anxi/depressive-like symptoms, the present study highlighted 5-HT4 receptor agonists as a promising antidepressive strategy that may also treat cognitive-associated symptoms in MDD. Since 5-HT4 receptor density does not decrease with age, it may also represent a new strategy to treat cognitive deficits in elderly patients with or without MDD.

Disclosures

DJD serves as a consultant for Lundbeck, Roche, and Servier. All other authors report no biomedical financial interests or potential conflicts of interest.

Acknowledgements

This work was supported by the technical assistance of Valerie Dupont-Domergue and the staff of the animal care facility of the SFR-UMS Institut Paris Saclay Innovation Thérapeutique. This work was supported by the Brain & Behavior Research Foundation (formerly NARSAD) and the Pierre Deniker Foundation (DJD), by the Ministère de l’Éducation Nationale, de l’Enseignement Supérieur et de la Recherche (MENESR, Paris, France) Fellowship (FD). We thank Pr Rene Hen (Columbia University) for insightful comments on this project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2016.01.055.

References


[26] G. Vas, V. Coman, Y. Charnay, R.L. Neve, E.J. Nestler, J. Bockaert, M. Barrot, G. Debonnel, Frontocortical 5-HT4 receptors exert positive feedback on

[27] G. Vas, V. Coman, Y. Charnay, R.L. Neve, E.J. Nestler, J. Bockaert, M. Barrot, G. Debonnel, Frontocortical 5-HT4 receptors exert positive feedback on
and the U.S.A.